J Young Pharm, 2018; 10(1): 37-40
A multifaceted peer reviewed journal in the field of Pharmacy
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Original Article

Formulation and Characterization of Rice Bran Oil in Alginate Microcapsules

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ABSTRACT

Objective: This study was conducted to evaluate the encapsulated Rice Bran Oil with sodium alginate to improve stability and reduce rancidity by using ionic gelation method. **Method:** Response Surface Methodology design were used to optimized microcapsule condition. Thirteen formula of Rice Bran Oil microcapsules were prepared in differential ratio and evaluated for physical characteristic, level of γ-oryzanol content, entrapment efficiency, in vitro release of Rice Bran Oil microcapsules. **Results:** Between all formulas, the ratio of 40:50:10 for Rice Bran Oil, sodium alginate and emulgator respectively, had the highest entrapment efficiency and in the physical characteristic parameter. The microcapsules were in particle range of 580 and 747μm. the in vitro evaluation showed that RBO was release from microcapsules in intestinal simulated digestion (50.64%) for 8 hours. **Conclusion:** Rice Bran Oil, sodium alginate and emulgator with the ratio of 40:50:10 indicated establish formula to create RBO microcapsules.
Key words: Formulation, Rice Bran Oil, Microcapsules, Ionic gelation, Sodium alginate.

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DOI: 10.5530/jyp.2018.10.10

INTRODUCTION

Rice milling process produces a major co-product, such as rice bran. Even though the rice bran it's a natural resource of fiber (8-10%), protein (14-16%), fat (12-23%), vitamin, and leading phenolic and unsaturated fatty acid and also it can be prepared to rice bran oil. Rice bran oil is a unique edible oil and a natural resource for powerful bioactive phytochemicals. Rice bran oil had antioxidant compounds such as tocotrienol, tocopherol, and γ-oryzanol.[1] Gamma γ-oryzanol is majorly compound ester trans-ferulic acid, phytosterol, β-sitosterol, 2,4 cycloartenol and campesterol.[2] Antioxidant activity, anti-cholesterol activity, and also gastroprotective effect are the utility of Gamma γ-oryzanol.[3] Unfortunately, this natural resources appears an endogenous enzyme (peroxidase, lipoxygenase, and lipase) which is result of microbial activity during the milling process, and when there is no further process to stabilization or storage of rice bran in room temperature around 6 hours or for long time period, its lead to degradation and transformation of odor and flavors and certainly uncomfortable for human application.[4,5] Microencapsulation is a technology to create a thin film round over the core particle to subtract the core interaction with uncertain environment and obviates chemical reaction.[6] This is an effective tactic to resist the environment situation. Many techniques have been constructed to shape microgel of oil microcapsules, for example, extruction, melt injection, and spray drying. Extraction has been reported to be an attractive method for producing oil microcapsules. The core and polymer material are simultaneously pumped through the needle.[7] Alginate is the most suitable natural polymer containing α-L-glucuronic acid (G) and 1,4-β-D-mannuronic acid (M) which obtained from brown seaweed. Thus, its less toxicity, good compatibility, long-term stability and easy to controlling gelation process.[8]

This research was aimed to investigate the optimum condition of rice bran oil microcapsules with different ratio of the core material and to evaluate the characteristic of RBO microcapsules.

MATERIAL AND METHODS

Materials

Fresh rice bran was provided by rice grinding in South Bogor Indonesia. Sodium alginate was obtained by Buchi (Switzerland). The γ-oryzanol standard was procured from Sigma-Aldrich (Missouri, USA), tween 20, span 20 were purchased from Merck (German). All analytical or HPLC grade reagents and solvent were supplied by Merck (German).

Extraction technique of rice bran oil

Fresh rice bran was stabilized according to heat process previously described by Noppawat *et.al.*[910] Briefly, rice bran was stored in an oven at 105°C for 15 minutes. These proceeds follow different steps, including extraction by cold pressed extraction, stirring, freezing and evaporating.

Preparation of rice bran oil microcapsules

Rice Bran Oil (RBO) microcapsules were prepared by ionic gelation method using alginate as a polymer. The microcapsules were prepared using Response Surface Methodology (RSM) to optimize the optimum condition of RBO microcapsules. The design consisted of 13 experimental points which included replicated center points and a set of a point lying at the midpoints of each edge of the multidimensional cube that defines the region of interest.[11] 1.5% Sodium alginate solution was roughly

mixed with RBO and composite emulgator (tween 20 and span 20) using magnetic stirrer for the certain time. Hereafter, the emulsion was sprayed into a beaker containing 3% calcium alginate solution. The droplet was collected and washed three times with 50 ml distilled water and dried at room temperature. The microcapsules could be dried within 24 h.[12]

Chromatogram analysis of γ-oryzanol

The γ-oryzanol concentration was confirmed based on the report of Masaralo et al. (2017) with slight modification using high-performance liquid chromatography (Shimadzu Corp., Japan) and equipped with Zorbax Eclipse plus C-18 Analytical 4.6 x 150 mm, (Aligent Technologies-USA). The eluent mixture was accomplished with acetonitrile: methanol: acetic acid (50:40:10) at flow rate 1.0 ml/min and detection was performed at a wavelength of 315 nm.[13]

Microcapsules evaluation

Entrapment efficiency of microcapsules

The RBO microcapsules were extracted with 10 ml hexane followed by ultrasonication in water for 90 minutes. The solutions were centrifuged (Universal 320 Hettich Zentrifugen, Germany) at 1372 G for 10 min. A volume of 1 ml supernatant was diluted with hexane and filtered through microphore Whatman 0.45 µm syringe filter and measured using HPLC analysis. The encapsulation efficiency of γ-oryzanol was determined by the Following equation[14]:

$$EE = \frac{Entrapped\ quality\ of\ RBO\ microcapsule}{Initial\ quality\ of\ RBO\ microcapsule} x\ 100$$

Particle size distribution

The diameter and surface characteristics of RBO microcapsules were determined by the optical microscope (Nikon Eclipse E200) with a magnification of 100x. In all measurements at least 100 particles were examined. The optical microscope was validated first before observation. Ocular micrometer scale was aligning with micrometer slide then measure the space. The measurement results obtained scale 7 adjacent to 0 whereas the scale 10 adjacent to 12. The space between 7-10 is 300 µm.[15]

SEM analysis

The surface morphology of the RBO microcapsules was investigated with scanning electron microscope for examining the surface morphology. The microcapsules were mounted on the metal grid with a double-sided adhesive tape, coated with gold under vacuum.[16]

In vitro release studies

The release of γ-oryzanol from RBO microcapsule was investigated using dissolution instrument (Electrolab TDT-08L USP) in two digestive liquid models as follows: 0.05 M HCl (pH 1.2) as stomach liquid models and 0.05 M phosphate buffer solution (pH 7.4) as intestine liquid models. 0.5% tween 80 was added to the both of digestive liquid models to increase the solvability of γ-oryzanol. About 1 gram of RBO microcapsules was weighed then dissolved in 900 ml digestive liquid models. The liquid samples at time intervals were evaluated by HPLC. The formula of fractional release was determined as follows:

$$fractional\ release: \frac{Mt}{Ma} x\ 100$$

Where Mt is the quantity of γ-oryzanol released from microcapsules at a certain time and Ma is the quantity of γ-oryzanol initially entrapped in the microcapsules. All experiments were performed in triplicate and values are expressed means and standard deviation.

RESULTS

Rancidity is a major problem to exploit potential purpose of rice bran. There are germ and outer stratum of cryopsis which have enzyme system (lipase, lipoxygenase, and peroxidase) that effect to the quality and rice bran characteristic. Lipoxygenase and lipase can cause lipid peroxidation and lead to rice bran degradation. To avoid rice bran from derivation, its required for stabilization after grinding procedures.

Chromatogram analysis of γ-oryzanol

The major component of γ-oryzanol evaluated could verify that they were present in all samples with the same retention time of γ-oryzanol standard. HPLC chromatograms of γ-oryzanol shown in Figure 1. five peaks were detected in the chromatogram of γ-oryzanol standard and RBO and the retention time of the γ-oryzanol was around 27 min. The 2.4 methylene cycloartenyl ferulate compound was predominant in this samples.[17] According to Goufo et al that 2.4 methylene cycloartenyl ferulate content was 34-44%, cycloartenyl ferulate 19-26%, campesteryl ferulate 15-23%, β-sitosteryl ferulate 7-17% and stigmasteryl ferulate 1-7%.[18] The results were compared to the data reported by Moon et al. as a result, all peak were identified.[19]

Microcapsules evaluation

Entrapment efficiency of RBO microcapsules

A Box-Benkhen design was used in this study to optimize the condition of RBO microcapsules. Figure 2 shown the 3D surface of optimization RBO microcapsules. The optimum condition based on the value of γ-oryzanol was in R12. With composition RBO: alginate:emulgator (40:50:10). The statistical analysis showed that the model F-value of 8.09 implies the model was significant. Table 1 shows that the result of entrapment efficiency RBO microcapsules was affected by RBO content.

Particle size distribution

The average of diameter microcapsules is shown in Figure 3. The diameter microcapsules were counted by a micrometer. The range of RBO microcapsules was 480-747 µm. The optical micrograph of RBO microcapsules is shown in Figure 4. The median of RBO microcapsules was 643.5±41.67 µm.

Table 1 : Optimization of RBO microcapsules

Formulation	γ-oryzanol content (µg/ml)	EE (%)
R 1	1.62	99.02
R 2	1.97	98.67
R 3	1.64	98.99
R 4	1.47	99.13
R 5	2.54	98.23
R 6	2.26	98.45
R 7	2.44	98.03
R 8	1.33	98.93
R9	2.54	98.24
R 10	2.70	97.80
R 11	3.02	97.52
R 12	2.32	99.20
R 13	1.92	98.58

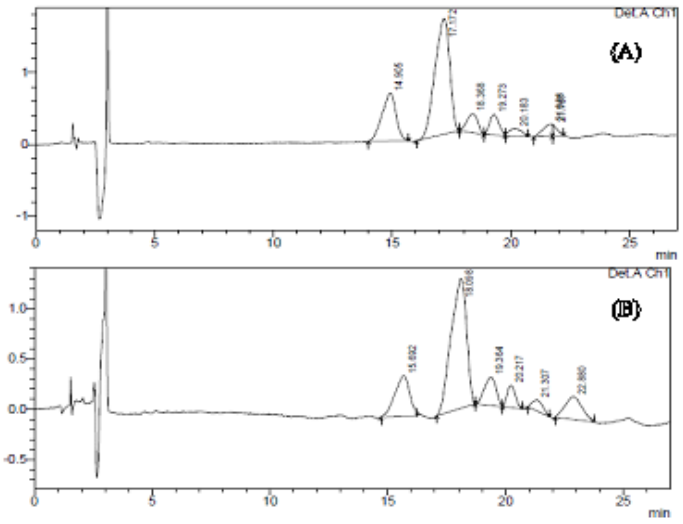

Figure 1: Representative HPLC of γ-oryzanol standard (A) and Rice bran oil (B).

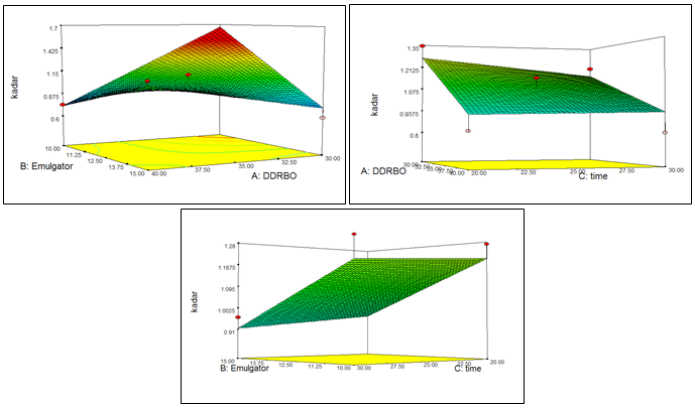

Figure 2: 3 D Response surface plot showing the effect of different RBO and coating time

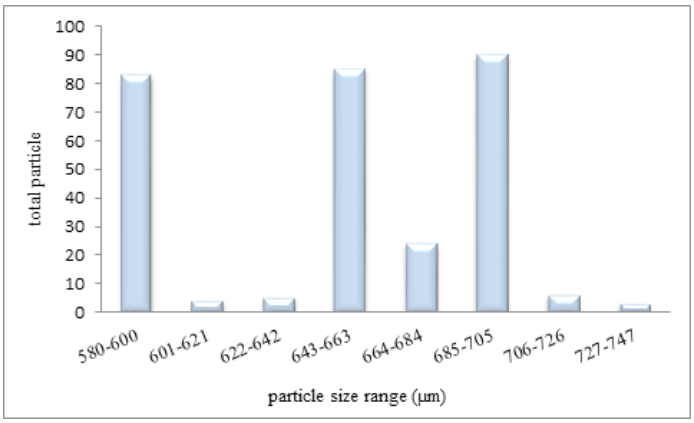

Figure 3: Particle size range of RBO microcapsules.

Scanning Electron Microscope (SEM) analysis

The SEM graph of RBO microcapsules are shown in Figure 5. The figure exhibited that the surface of this sphere had indention textural characteristic.

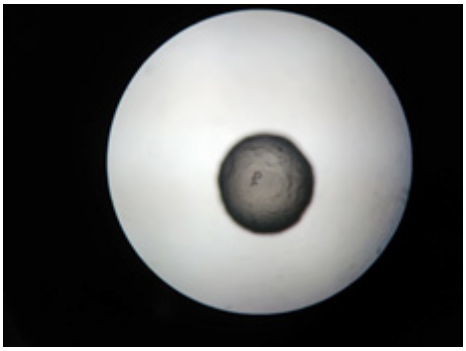

Figure 4: Optical graph of RBO microcapsules with 10x magnification.

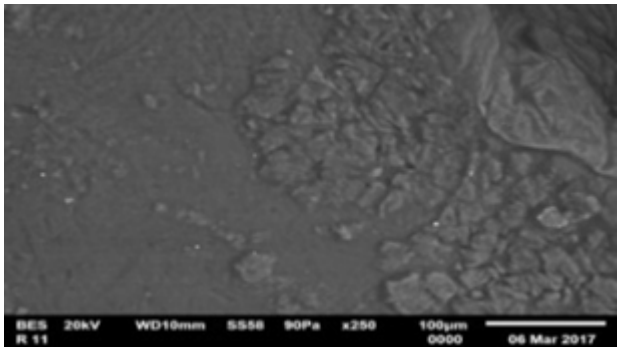

Figure 5: SEM graph of RBO microcapsules with 500x magnification.

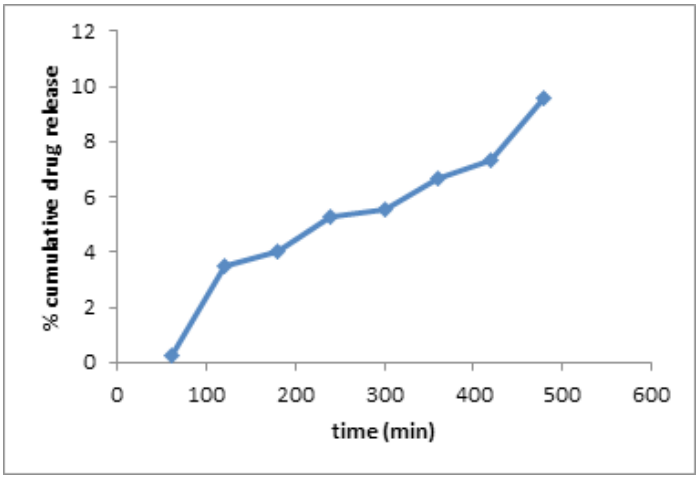

Figure 6: *In vitro* dissolution profile of RBO microcapsules in simulated gastric fluids.

In vitro release of RBO microcapsules

Figure 6 and 7 illustrated the in vitro release profile of RBO microcapsule in the simulated digestive fluid. In simulated gastric fluids (SGF), about 9.55% of the entrapped γ-oryzanol was released during the initial 8 h and only about 50.64% of the entrapped γ-oryzanol was released within 2 h in simulated intestinal fluid (SIF).

DISCUSSION

Gamma oryzanol is a biomarker for RBO. Its shown in Figure 1. The γ-oryzanol content of all formula is between 1.33-3.02 µg/ml. Response

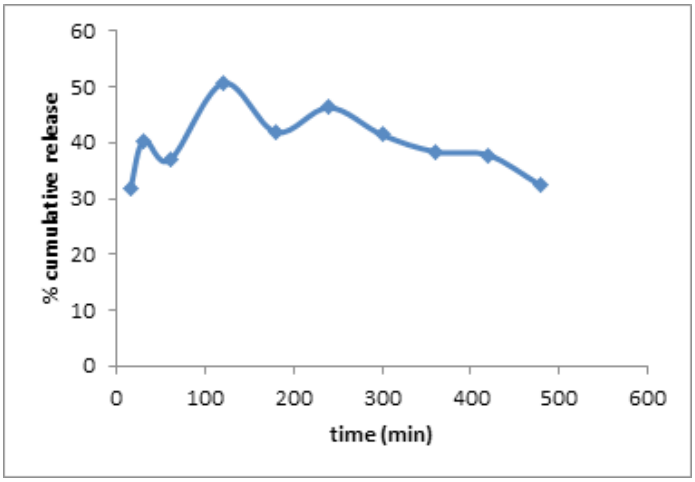

Figure 7: *In vitro* dissolution profile of RBO microcapsules in simulated intestine fluids.

surface methodology is used to optimize the condition of RBO microcapsules.[20] The run 12 shown the highest entrapment efficiency (EE) (40 part of RBO, 50 part of alginate, 10 part of emulgator). By adding more part alginate, the EE was increased to 99,19%. These explanations are prospered by Jeevana et al (2009). This research reported that increasing 3.8% gelatin solution, EE increased from 14.9% to 97.6%.[21] SEM graph explained that the alginate has been successful bind with calcium ion. This result in tune with Hosseini research depends on the loading oil into microcapsules.[22] The optical graph shown that RBO microcapsules were rough with some visible cracks. A good shape indicated that microcapsule formed a reticulated structure when contacted with calcium ions. The in vitro evaluation using USP dissolution for 8 hours. Based on in vitro data, the higher cumulative percentage release of R12 was 50.65% in 2 hours. the Ca ion in the crosslinked network could be released by ion exchange with sodium in SIF. The presence of crosslinking between alginate and calcium ions indicate the release of oryzanol in SIF. As reported by Singh et al, the release of pioglitazone was slow at SGF.[23]

CONCLUSION

The optimization of RBO microcapsules using RSM has been demonstrated. The results of this study provided that the optimum condition of RBO microcapsules are 40:50:10 (RBO: Sodium alginate:emulgator).

ACKNOWLEDGEMENT

This study financially was supported by Hibah PITTA 2017 from Directorate of Research and Community Engagement, Universitas Indonesia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Patil SS, Kar A, Mohapatra D. Food and Bioproducts Processing Stabilization of rice bran using microwave : Process optimization and storage studies. Food Bioprod Process. 2016;99:204–11.
2. Lerma-García MJ, Herrero-Martínez JM, Simó-Alfonso EF, Mendonça CRB, Ramis-Ramos G. Composition, industrial processing and applications of rice bran Y-oryzanol. Food Chem. 2009;115:389–404.
3. Kim JS, Lee JS, Chang PS, Lee HG. Optimization, in vitro release and bioavailability of gamma-oryzanol-loaded calcium pectinate microparticles reinforced with chitosan. N Biotechnol. 2010;27(4):368–73.
4. Capellini MC, Giacomini V, Cuevas MS, Rodrigues CEC. Rice bran oil extraction using alcoholic solvents_ Physicochemical characterization of oil and protein fraction functionality. Ind Crop Prod. 2017;104:133–43.
5. Thanonkaew A, Wongyai S, McClements DJ, Decker EA. Effect of stabilization of rice bran by domestic heating on mechanical extraction yield, quality, and antioxidant properties of cold-pressed rice bran oil (Oryza saltiva L.). LWT - Food Sci Technol. 2012;48(2):231–6.
6. Gangurde AB, Sav AK, Javeer SD, Moravkar KK, Pawar JN, Amin PD. Modified extrusion-spheronization as a technique of microencapsulation for stabilization of choline bitartrate using hydrogenated soya bean oil. Int J Pharm Investig. 2015;5(4):275–83.
7. Wu Q, Zhang T, Xue Y, Xue C, Wang Y. Preparation of alginate core–shell beads with different M/G ratios to improve the stability of fish oil. LWT - Food Sci Technol. 2017;80:304–10.
8. Eqbal MD, Gundabala V. Controlled fabrication of multi-core alginate microcapsules. J Colloid Interface Sci. 2017;507:27–34.
9. Pengkumsri N, Chaiyasut C, Sivamaruthi BS, Saenjum C, Sirilun S, Peerajan S, *et al*. The influence of extraction methods on composition and antioxidant properties of rice bran oil. Food Sci Technol. 2015;35(3):493–501.
10. Liu M, Yang F, Shi H, Akoh CC, Yu L. Preparative separation of triterpene alcohol ferulates from rice bran oil using a high performance counter-current chromatography. Food Chem. 2013;139(1–4):919–24.
11. Lee J-S, Kim JS, Lee HG, Ji-Soo L, Kim JS, Lee HG. Gamma-oryzanol-loaded calcium pectinate microparticles reinforced with chitosan: optimization and release characteristics. Colloids Surf B Biointerfaces. 2009;70(2):213–7.
12. Soliman EA, El-moghazy AY, El-din MSM, Massoud MA. Microencapsulation of Essential Oils within Alginate : Formulation and in Vitro Evaluation of Antifungal Activity. 2013;2013(March):48–55.
13. Massarolo KC, Ribeiro AC, Furlong EB. Effect of particle size of rice bran on gamma-oryzanol content and compounds. 2017;75:54–60.
14. Boggione DMG, Batalha LS, Gontijo MTP, Lopez MES, Teixeira AVNC, Santos IJB, et al. Evaluation of microencapsulation of the UFV-AREG1 bacteriophage in alginate-Ca microcapsules using microfluidic devices. Colloids Surfaces B Biointerfaces. 2017;158:182–9.
15. Vedha HB, Brahma RA, Samyuktha RB. Floating drug delivery of nevirapine as a gastroretentive system. J Young Pharm. 2010;2(4):350–5.
16. Kim JS, Lee JS, Chang PS, Lee HG. Optimization, in vitro release and bioavailability of ??-oryzanol-loaded calcium pectinate microparticles reinforced with chitosan. N Biotechnol. 2010;27(4):368–73.
17. Lu W, Niu Y, Yang H, Sheng Y, Shi H, Yu L. Simultaneous HPLC quantification of five major triterpene alcohol and sterol ferulates in rice bran oil using a single reference standard. Food Chem. 2014;148:329–34.
18. Goufo P, Trindade H. Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ -oryzanol, and phytic acid. Food Sci Nutr. 2014;2(2):75–104.
19. Moon S-H, Kim D, Shimizu N, Okada T, Hitoe S, Shimoda H. Ninety-day oral toxicity study of rice-derived γ-oryzanol in Sprague-Dawley rats. Toxicol Reports. 2017;4:9–18.
20. Ayuningtyas Imalia, Rahmawati Maesya, Sutriyo MA. Optimization of Ionic Liquid-Based Microwave Assisted Extraction to Obtain Trans-resveratrol from Gnetum gnemon L.Seeds. J Young Pharm. 2017;9(4):457–62.
21. Jeevana J, Sunitha G. Development and evaluation of gelatin microspheres of tramadol hydrochloride. J Young Pharm. 2009;1(1):24.
22. Hosseini SM, Hosseini H, Mohammadifar MA, Mortazavian AM, Mohammadi A, Khosravi-Darani K, et al. Incorporation of essential oil in alginate microparticles by multiple emulsion/ionic gelation process. Int J Biol Macromol. 2013;62:582–8.
23. Singh C, Jain K, Kumar C, Agarwal K. Design and *in vitro* evaluation of mucoadhesive microcapsules of pioglitazone. J Young Pharm. 2009;1(3):195.

Article History: Submission Date : 14-11-2017; Revised Date : 21-11-2017; Acceptance Date : 13-12-2017.
Cite this article: Nashihah S, Mun'im A, Sutriyo, Saputri FC. Formulation and Characterization of Rice Bran Oil in Alginate Microcapsules. J Young Pharm. 2018;10(1):37-40.